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Installation Restoration Research Program

Base-Induced 2,4,6-Trinitrotoluene Transformation, Titration Studies

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14. ABSTRACT
The removal of 2,4,6-trinitrotoluene (TNT) from solution by the addition of a base, sodium hydroxide (NaOH), was examined. TNT solutions were titrated to determine the required hydroxide concentration for the removal of TNT from solutions to proceed. It was found that the pH required for removal of half the TNT was 10.9 ± 0.18 with an empirical coefficient of 0.16 ± 0.11. A parametric analysis was performed, and the model was determined not to be sensitive with respect to the empirical coefficient.

This study found a near-instant removal of TNT when the pH of the solution was increased above 11. Several unidentified compounds were formed sequentially. The sequence of these unidentified compounds appears to indicate an increase in their polarity. The addition of NaOH resulted in a reduction in the acute toxicity of TNT solutions based on Microtox® tests with *Vibrio fischeri.*

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Preface

The work reported herein was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, as part of cleanup technology development supported by the Environmental Quality Technology Program, Project AF25-302E.

This report was prepared by Mr. Lance Hansen of the Environmental Engineering Branch (EEB), Environmental Laboratory (EL), ERDC; Mr. David Ringelberg of the Environemental Sciences Branch of the Cold Regions Research and Engineering Laboratory (CRREL), ERDC; Ms. Deborah Felt of Applied Research Associates, Inc. (Southern Division), and Dr. Jeffrey Davis of the Environmental Engineering Branch (EEB), EL, ERDC. Chemical analyses were performed by the Environmental Chemistry Branch, EL, ERDC.

We gratefully acknowledge the support provided by Dr. John Cullinane, EL, ERDC. The study was conducted under the direct supervision of Mr. Danny Averett, Chief, EEB, and Dr. Richard E. Price, Chief, Environmental Processes and Engineering Division, and under the general supervision of Dr. Edwin A. Theriot, Acting Director, EL.

At the time of publication of this report, Director of ERDC was Dr. James R. Houston, and COL John W. Morris III, EN, was Commander and Executive Director of ERDC.

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1 Introduction

The explosive 2,4,6-trinitrotoluene (TNT), as an environmental contaminant, is largely the result of munitions manufacturing. As of 1985, 2 million pounds per year of TNT was being produced in the United States (Harter 1985). Tsia (1991) reported that during both world wars, TNT was produced in enormous quantities, generating an average of 80,000 gal of explosives-contaminated water and 250,000 lb of solid waste per day. Although TNT production in the United States has since been drastically reduced, environmental TNT contamination remains a significant problem (Heilman,Wiesmann, and Stenson 1996). During loading, transporting, and handling, TNT was spilled, dumped, or otherwise released into the environment in large quantities. These incidents have resulted in contaminant concentrations as large as 10,000 ppm in soil and 100 ppm in water (Fernando, Bumpus, and Aust 1990). Additionally, cleaning and repacking of old munitions continues to generate large continuous flows of TNT-contaminated industrial wastewater (Boopathy et al. 1994).

The U.S. Environmental Protection Agency (EPA) lists TNT as a priority pollutant and has recommended its removal from contaminated sites. It has been advised that the maximum allowable TNT concentration in drinking water be set at 140 μg/L. The EPA lifetime health advisory for TNT in drinking water is 20 μg/L (Rosenblatt et al.1991, Roberts and Hartley 1992). TNT may have a significant detrimental impact on the environment because of its toxicity towards algae, invertebrates, and fish (Won, DiSalvo, and Ng 1976, Bradley and Chapelle 1995) and the growth inhibition seen in fungi, bacteria, and plants (Bradley and Chapelle 1995, Spiker, Crawford, and Crawford 1992, Palazzo and Leggett 1986, Simini et al. 1995).

Won, DiSalvo, and Ng (1976) studied the effects of TNT on unicellular green algae, tidepool copepods, oyster larvae, and *Salmonella typhimurium*. They found that TNT and several of its metabolites in concentrations as low as 2.5 ppm caused some ill effects, and concentrations as low as 5 ppm were toxic and/or mutagenic. White rot fungus has been known to degrade TNT by its non-specific extracellular peroxidases, but TNT is toxic to this organism above 24 ppm in the soil (Spiker, Crawford, and Crawford 1992). Bacterial consortia that had not been previously exposed to TNT showed toxic effects at TNT concentrations as low as 0.23 ppm. Bacterial consortia isolated from contaminated sites showed up to 11 percent mineralization of TNT in concentrations up to 113 ppm, but TNT was toxic to the consortia at concentrations above that level.

Toxicity studies have also indicated that TNT has adverse effects on humans and laboratory test animals. TNT and its metabolites are considered to be both mutagenic and carcinogenic, Class C (Rosenblatt et al. 1991, ASTDR 1995, Jarvis, McFarland, and Honeycutt 1998).

The study of TNT degradation has interested researchers for many decades. Since the mid-1970s, beginning with McCormick, Feeherry, and Levinson (1976), biotransformation of TNT has been of significant interest. Since then, many researchers have attempted to evaluate various mechanisms of TNT biodegradation and optimize TNT biodegradation under various conditions of nutrient and substrate amendment, pH control, Eh control, bioaugmentation, and surfactant addition. Experimental results have varied drastically. Consequently, bioremediation of TNT-contaminated soil, sediments, and water is still considered innovative and is not widely accepted by regulatory officials overseeing the cleanup of explosives-contaminated sites.

More recently it has been recognized that abiotic reduction may provide mechanisms to transform not only parent contaminants, but also toxic products generated by reductive transformation of the parent compound. An abiotic reduction of organic pollutants in reducing environments has drawn considerable attention.

The transformation of TNT in basic solutions has long been established. Urbanski (1964) described a reddish brown solution after the addition of base, but no products or mechanisms were identified. Saupe, Garvnes, and Heinze (1997) conducted ex situ flask experiments with high alkaline attack (pH 14), resulting in complete TNT transformation and partial mineralization. The intent of this work was to follow chemical pretreatment with thermal treatment for destroying residual byproducts. Saupe suggested that alkaline hydrolysis may be an effective treatment when coupled with another technology (e.g. biological remediation).

Several technologies for TNT degradation were enhanced at elevated pH. Dillert et al. (1995) noted that at pH 11, the rate of TNT transformation with ultraviolet light was as fast as if a TiO_2 catalyst were present. Brannon, Price, and Hayes (1998) reported that at pH 8, the TNT transformed faster than at pH 6 or 7 when exposed to Fe(II). TNT degradation by a combination of UV light, ozone, and electrohydraulic discharge was enhanced at and above pH 5 (Lang et al. 1998). Degradation intermediates of TNT were polymerized in alkaline conditions during anaerobic bioremediation of soils (Funk et al. 1993). Dunnivant and Schwarzenbach (1992) reported that TNT degradation caused by natural organic matter was increased at elevated pH.

Saupe, Garvnes, and Heinze (1997) studied the alkaline hydrolysis of TNT at elevated temperatures. Adding sodium hydroxide to TNT solutions created a dark brown hydrosylate. Above 60 °C, polymerization was evident, with increased molecular size in the dissolved organic fraction. Solids were formed instead of mineralization at and above 200 °C with this method. A Meisenheimer or charge transfer complex was postulated. The authors suggested a treatment of

80 °C with sodium hydroxide, followed by a biotreatment technology after neutralization.

Arienzo (1999) found that TNT was completely removed from soil in 10 min with the application of 1 percent calcium hydroxide, $Ca(OH)_2$. In a recent study, Emmrich (1999, 2001) also treated TNT solutions and TNT-contaminated soils with calcium hydroxide at 20 °C. Aqueous samples were extracted with ethyl acetate, brought to dryness, dissolved in methanol, and analyzed for TNT by gas chromatography. Nitrite and nitrate formation were also reported. TNT transformation was described by a psuedo-first-order rate constant with respect to TNT. An initial violet color was interpreted as the formation of a TNT anion.

A closer examination of the alkaline hydrolysis of TNT is required before this technology is ready to use for remediating contaminated soils or water. This paper describes a study of the chemical and toxicological effects of increased pH on TNT-contaminated water. This research also provided insight into the kinetics and stoichiometry of the hydrolytic reaction.

2 Materials and Methods

Chemistry and Toxicology of the OH/TNT Reaction

A 100-mL TNT solution (86.8 mg/L) was titrated with 2-mL aliquots of 1-M KOH over a pre-determined time course (1, 5, 15, and 30 min, 1, 3, 5, 7, 22, 30, and 48 hr). Immediately following each titration event, 2-mL aqueous samples were collected and analyzed for TNT using a modified EPA method 8330. The pH was recorded (after three-point calibration) following each titration event during sample collection.

The toxic effect of hydroxide treatment was determined by adding sodium hydroxide to water contaminated with TNT at 10 mg/L and 100 mg/L. Sodium hydroxide was added to 2 mM and 4 mM in each TNT solution. Acute toxicity was determined for the untreated solution and each treated solution according to the standard Microtox® procedure, *Microtox® Acute Toxicity (Condensed) 3 Samples Simultaneously Test* protocol, 1995 (Azur Environmental). The results are reported as EC_{50} values, the effective concentration where 50 percent of the expected fluorescence from the test bacterium, *Vibrio fischeri*, is inhibited. Higher EC_{50} values indicate lower acute toxicity.

Reaction Kinetics and Stoichiometry

To evaluate the effect of time on the hydrolytic reaction of TNT, several buffers were made; their composition is shown in Table 1. Twenty milliliters of each buffer was added to flasks containing 80 mL of 21.5-mg/L TNT solution. After incubating for 1 min and 24 hr, aliquots of the buffered solution were taken and neutralized with 0.1-N HCl. Samples were analyzed immediately for TNT concentration.

To determine the pH required to remove TNT, four of the buffers shown in Table 1 (pH levels 7, 8, 9,and 10) were inoculated with TNT to 24 mg/L in triplicate. These solutions were titrated at 1-min intervals by adding 1 mL of 0.1-N NaOH, stirring the solution for 1 min, and removing and neutralizing a 1-mL aliquot for TNT analysis.

Table 1
Buffer Composition

Initial pH Value	Buffer Composition
7	50 mL 0.1-M KH_2PO_4, 29.1 mL 0.1-N NaOH, 20.9 mL H_2O
8	50 mL 0.1-M KH_2PO_4, 46.7 mL 0.1-N NaOH, 3.3 mL H_2O
9	50 mL of 0.1-M H_3BO_3, 20.8 mL 0.1-N NaOH, 29.2 mL H_2O
10	50 mL $NaHCO_3$, 10.7 mL 0.1-N NaOH, 39.3 mL H_2O
11	50 mL 0.05-M $NaHCO_3$, 22.7 mL 0.1-N NaOH, 27.3 mL H_2O

Instrumentation

Explosives analysis was performed using a Hewlett Packard 1090 with diode array detector (DAD) at 254 nm. A C-18 reverse-phase column and an isocratic mobile phase of 68% methanol/ butanol mixture (98:2) and 34% 20-mM ammonium chloride solution were used.

3 Results and Discussion

Chemistry and Toxicology of the OH/TNT Reaction Products

The complete removal of TNT (86.6 mg/L) occurred within 1 min after titration with 2 mL of 1-N NaOH. The pH level of the solution was measured after each addition. The pH level increased from an initial value of 6 to a value of 12 after 1 min, and this pH value was then sustained throughout the experiment. Several unidentified products were produced after continued titration; their production and disappearance are shown in Figure 1. The spectra of these transformation products are shown in Figure 2.

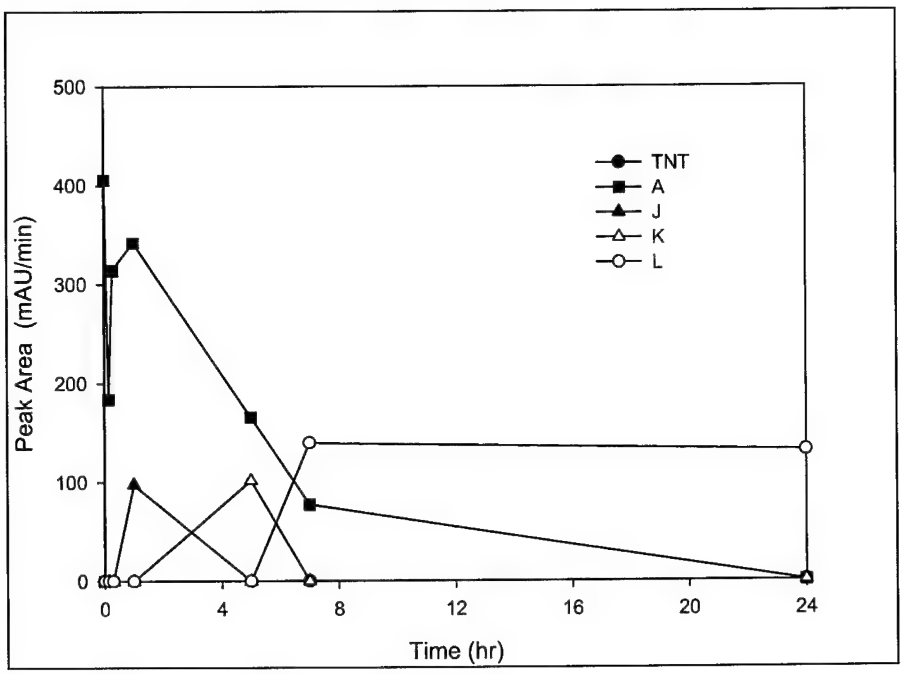

Figure 1. TNT transformation product formation and disappearance

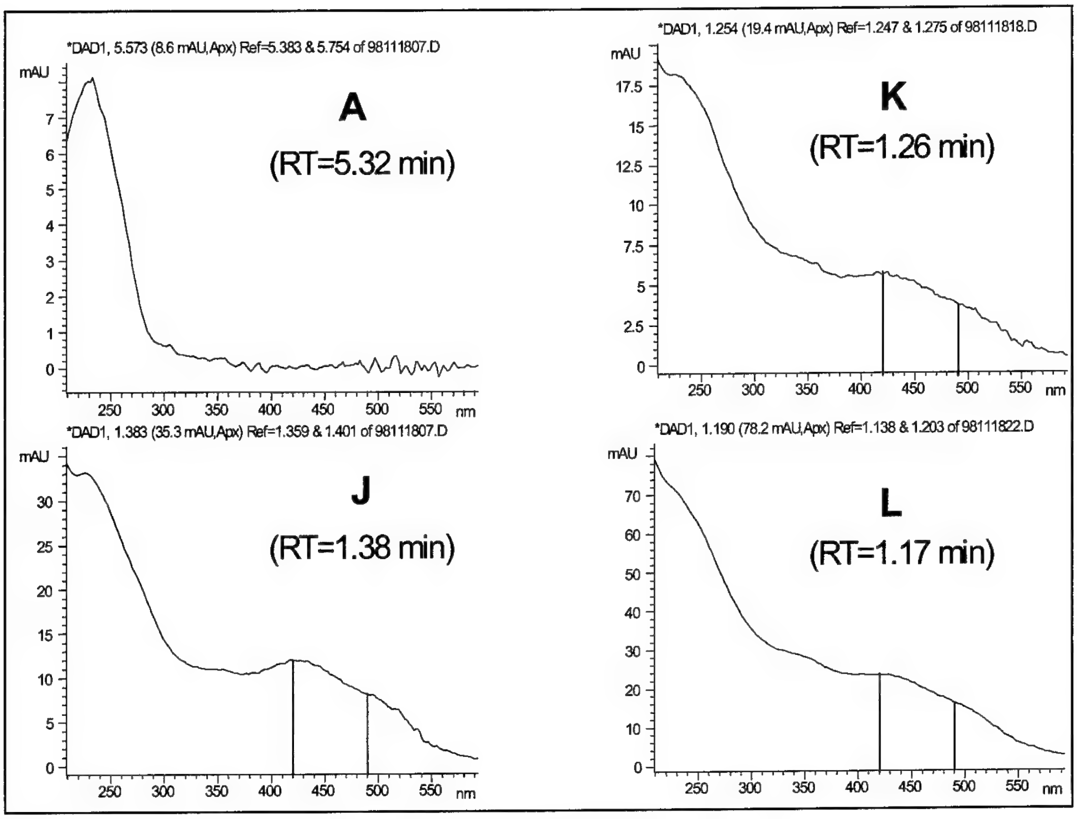

Figure 2. UV spectra of unidentified intermediates

A closer examination of Figure 1 reveals a succession of the unknown intermediates schematically represented by:

$$\mathrm{TNT} \rightarrow \mathrm{A} \rightarrow \mathrm{J} \rightarrow \mathrm{K} \rightarrow \mathrm{L}\,.$$

Table 2 shows the retention times for TNT and the unknown compounds. The reverse-phase column was used in the HPLC for separating compounds. Shorter retention times indicate increasingly polar compounds. The sequence shown above for the production and disappearance of TNT and unknown products, and the results summarized in Table 2, indicate that the reaction of hydroxide and TNT causes an increase in polarity. Studies are underway to identify these unknown compounds.

Table 2 HPLC Retention Times for TNT and Unknown Intermediates	
Compound	**HPLC Retention Time (min)**
TNT	5.94
Unknown compound A	5.32
Unknown compound J	1.38
Unknown compound K	1.26
Unknown compound L	1.17

Although TNT can demonstrably be removed from aqueous solutions by elevating the pH, it remained to be determined if the addition of base decreased TNT's toxicity. Figure 3 is a plot of the acute toxicity of two solutions (10 mg/L and 100 mg/L) of TNT after the addition of NaOH at concentrations of 0, 2, and 4-mM NaOH and an incubation period of 1 hr. The addition of NaOH decreased the toxicity of TNT at concentrations of 10 and 100 mg/L. These results show that the addition of hydroxide to TNT solutions is a rapid method for both removing TNT from solution and reducing the toxicity in that solution.

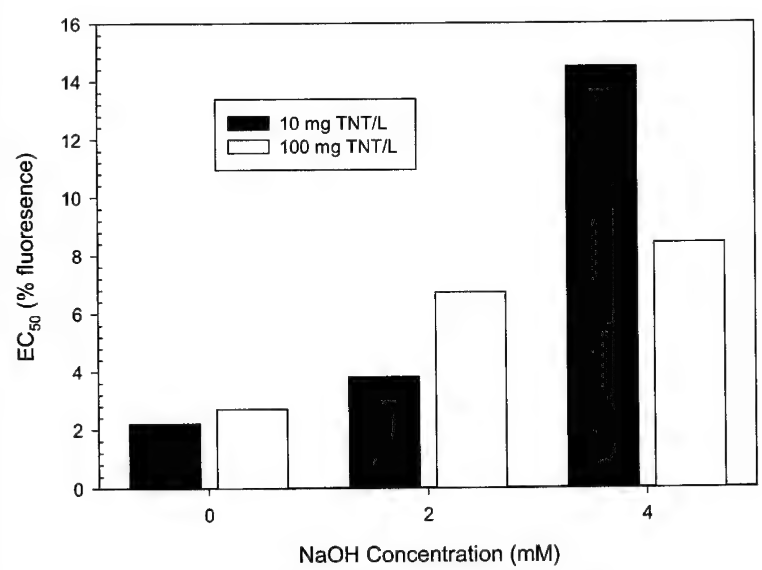

Figure 3. Acute toxicity of TNT solutions treated with NaOH

Reaction Kinetics and Stoichiometry

The reaction kinetics of TNT and NaOH were determined in a set of experiments conducted to examine the effect of pH and time on the removal of TNT from water. TNT was added to five buffered solutions of various pH values, and aliquots were removed after 1 min and 24 hr and examined for TNT concentration. The results of this experiment, as shown in Figure 4, indicate no significant difference between the samples removed after 1 min and 24 hr ($p = 0.05$). The results for effect of pH showed a small removal (36%) of TNT at pH 11, indicating a very rapid removal of TNT at elevated pH.

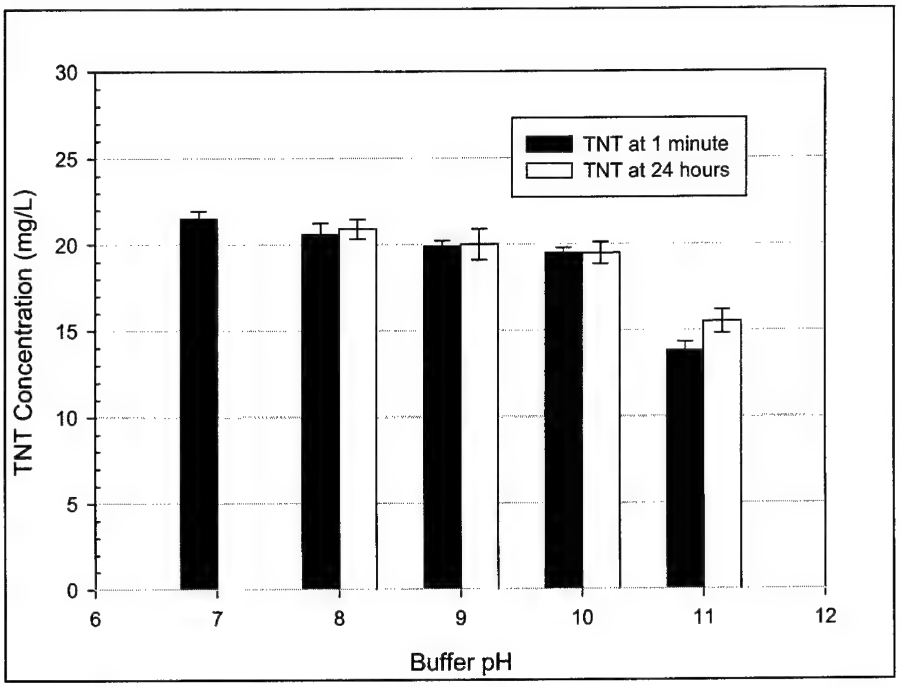

Figure 4. TNT concentrations in buffer solutions of various pH values as a function of time

Titrations were performed to examine the pronounced effects of pH on the removal of TNT from solution. The titrations were carried out in triplicate in buffered solutions at pHs of 7, 8, 9, and 10. The results of these titrations (Figure 5) follow similar patterns. Little or no removal of TNT was found at pHs below 10, and complete removal was found at pHs above 11.5.

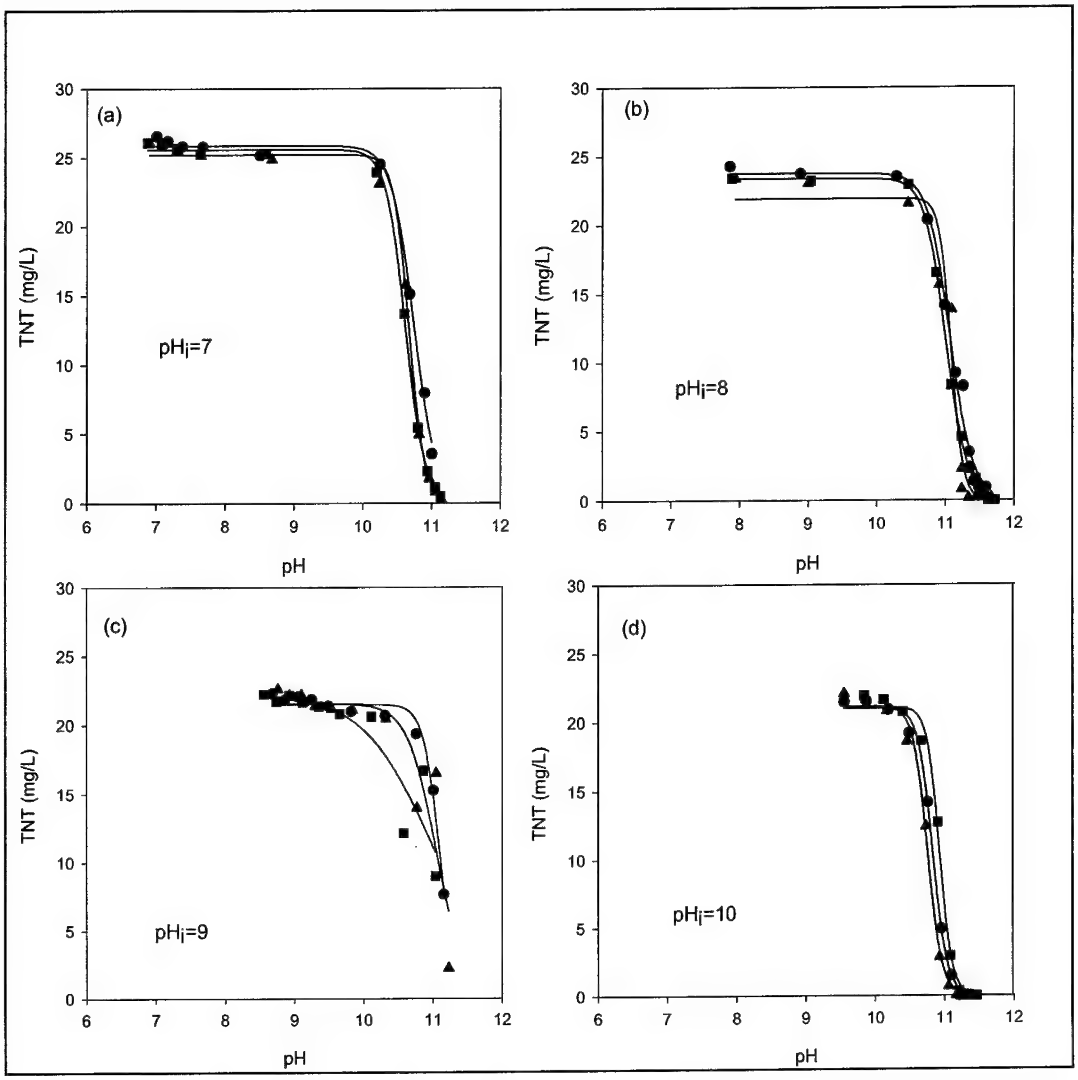

Figure 5. Titration curves of various buffered TNT solutions

Using a sigmoid function, a curve was fit to the data obtained from the titrations (Figure 5). The sigmoid function used is

$$C = \frac{C_0}{1+\exp\left[\frac{pH - pH_{1/2}}{b}\right]}$$

where

C_0 = initial TNT concentration (ppm)

$pH_{1/2}$ = pH at 50% TNT removal

b = empirical constant

The results of a regression analysis of the titration curves from Figure 5 are summarized in Table 3. This analysis yields a $pH_{1/2}$ of 10.9 ± 0.18, with an empirical coefficient of 0.16 ± 0.11. The large standard deviation associated with the empirical coefficient caused concern. A parametric analysis was performed to examine the sensitivity of this empirical coefficient. The results are show in Figure 6. A cursory examination of Figure 6 shows that over the range of values for b (0.086 to 0.502), little difference was found. Therefore this parameter is insensitive.

Table 3
Regression Analysis of Titration Curves of TNT

	C_0 (ppm)	$pH_{1/2}$	b	r^2
pH_i=7				
A	25.9	10.7	0.164	0.996
B	25.6	10.6	0.142	0.999
C	25.2	10.7	0.113	0.977
pH_i=8				
A	23.7	11.1	0.164	0.990
B	23.4	11.0	0.154	0.999
C	21.9	11.1	0.086	0.971
pH_i=9				
A	21.5	11.1	0.118	0.981
B	22.3	11.0	0.502	0.833
C	21.5	11.1	0.208	0.827
pH_i=10				
A	21.0	10.8	0.104	0.998
B	21.1	10.9	0.090	0.996
C	21.1	10.8	0.103	0.996
Mean	22.85	10.9	0.16	
Standard Deviation	1.85	0.18	0.11	

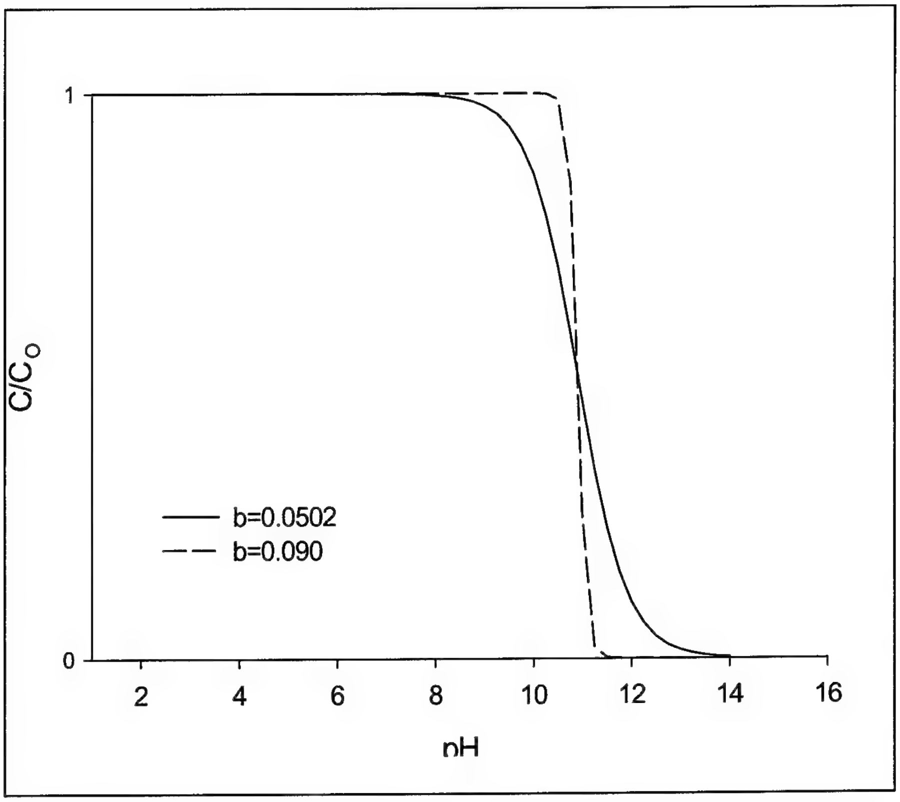

Figure 6. Parametric analysis of TNT removal model

4 Conclusions

This study provided valuable information concerning the efficacy of hydroxide addition for remediating TNT-contaminated water. TNT was completely removed from aqueous solutions at pHs above 11.5, and the addition of hydroxide to TNT solutions reduced toxicity.

Several questions will be addressed in further studies:

- What are products that are produced by the addition of hydroxide?
- Are other parameters (e.g., temperature, TNT concentration, counter-ion, etc.) significant in the removal of TNT?
- Will this method work for the remediation of TNT-contaminated soils?

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